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### **Fatty Acid Composition of the Marine Gastropods *Telescopium telescopium* and *Bursa spinosa* Collected from the Southwest Coast of India.**

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#### **ABSTRACT**

Fatty acid profiles of two marine gastropods, *Telescopium telescopium* collected from a mangrove estuary and *Bursa spinosa* from harbour area were determined. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs) and nonmethylene interrupted fatty acids (NMIs) were identified in the study. The main fatty acid components in *T. telescopium* were iC17:0, C16:0, C18:0, C18:1n9, C18:1n13, C20:1n5, C18:4n4, C22:4n6, C20:5n3 and C22:6n3. Out of this fatty acids hexadecanoic acid (C16:0) was dominant (20%). C16:0, C17:0, C18:0, C18:1n9, C22:2n6, C18:4n4 and C22:5n3 were the major fatty acids observed in *B. spinosa*. C22:5n3 was dominant (44%) in *B. spinosa*. Among the fatty acids in *T. telescopium*, saturated fatty acids were the major component, whereas in *B. spinosa* PUFAs were predominant. In *B. spinosa*, 55% of the total fatty acids constitute n3 fatty acid and 20% comprise MUFAs. Among the PUFAs C22:2n6, C18:4n4, C22:4n6, C22:5n3 and C22:6n3 were high. The concentration of nonmethylene interrupted fatty acids (NMIs) were high in *T. telescopium* as compared to *B. spinosa*

**KEYWORDS:** Fatty acids, Gastropods, *Telescopium telescopium*, *Bursa spinosa*.

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## INTRODUCTION

Molluscs are the second largest phylum in the marine world<sup>1</sup>. Their morphological and physiological features attract many investigators. The phylum Mollusca is divided into seven classes of which polyplacophora, gastropoda, bivalvia and cephalopoda are the largest and they exhibit a diversity of lipid and fatty acid components in both freshwater and marine species. Molluscs have had a tremendous impact on the Indian tradition and economy. Amongst molluscs, gastropods in particular occupy an important role in the commercial shell craft industry. A wide variety of species exists on land, freshwater and in the sea. Marine gastropod resources are exploited for various purposes and often go ignored, as they form only a minor component of marine fishery resources. Many species are exported for the purposes of manufacturing ornaments, curios and various other artefacts of commercial value. Women and children collect this gastropods and bivalves from shallow estuaries for nutritional food. In Kerala coast, especially in Neendakara and Fortkochi, gastropods have been exporting to many states for making manure, fish feeds and poultry feeds. Shells and shell crafts of gastropods are the major economy for the local peoples in Kanyakumari and Mahabalipuram (Tamilnadu) coast. Marine bivalves and gastropods are also rich sources of many biologically active compounds. Owing to their medicinal and other industrial properties, several species of gastropods and bivalves are traditionally fished for food and shell.

Depending on the taxonomic relations, environmental conditions, nutrient habits, food availability and also physiological conditions, different fatty acids have been found in molluscs. Lipid compounds and their compositions have been widely studied in marine bivalves; a few investigations have been done in marine gastropods. In recent years, poly unsaturated fatty acids (PUFAs) have been recognized as a good remedy for cardiovascular diseases. EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are the most common n<sub>3</sub> fatty acids observed in marine gastropods and these have strong anti- arrhythmic action on the heart. Gastropods are rich source of 18:1n-9, 20:4n 6, 20:5n-3 and 22:5n-3 fatty acids. EPA and DHA prevent the development of ventricular tachycardia and fibrillation. Compared with information on fatty acid composition in the marine gastropod is notably limited in the literature. The present study was carried out to analyse the fatty acid compositions of two marine gastropods *T. telescopium* and *B. spinosa*. Basic elucidation is made of the various fatty acid components present in these two species.

## MATERIALS AND METHODS

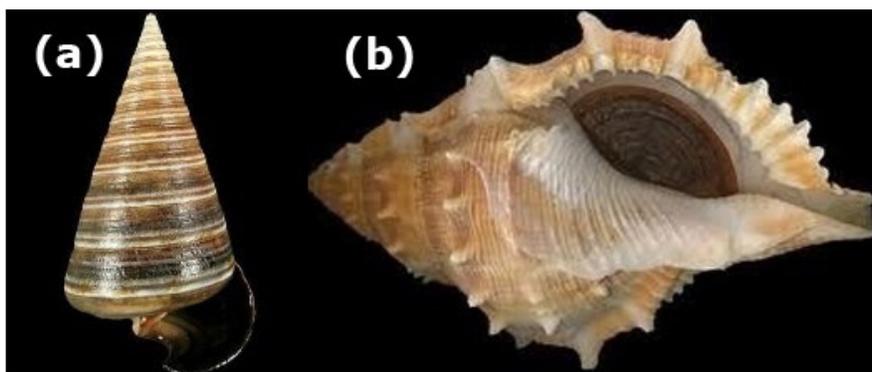
Mangrove snails *Telescopium telescopium* (Fig.1(a)) were collected from Pappinissery mangrove ecosystem (Latitude: 11° 56' 8" and Longitude: 75° 21' 13"), situated in Kannur district covering a distance of 7-8 km and shore snails *Bursa spinosa* (Fig.1(b)) were collected from the

coastline, Neendakara beach (Latitude: 8° 56' 40". Longitude: 76° 32' 25") situated in Kollam district of Kerala state. These two species were coming under the family mollusc (Table 1).

The collected samples were stored at -20<sup>0</sup> C until analysis. Extraction of fatty acids was carried out according to the slandered protocol developed by<sup>18</sup>. Extracted fatty acids were analysed as Fatty Acid Methyl Ester (FAME) in gas chromatography- mass spectrometry (GC-MS) (Perkin Elmer Clarus 620 GC), with MS detector equipped with a non polar HP ultra-double –fused silica capillary column (30m length, 0.32µm internal diameter, 0.25µm film thickness). Operating conditions were as follows: ion source of electron voltage 70eV kept at 200° C. Spectra were scanned from 50 to 600 m/z with scan time of 1.50 s. Initially, the temperature was increased from 50° C to 200° C at a rate of 2° C per min and held at 200° C for 5 min. Then, the temperature was again increased from 200°C to 280° C at a rate of 10°C per min and held at 280<sup>0</sup> C for 10 min. Full data acquisition was obtained with the use of MS Turbomass version 5.4.2. Quantification was achieved by calibration of FAMEs standards supplied by Sigma Aldrich (Supelco 37 Component FAME Mix, 18919-1AMP). Sample FAMEs were also injected under the above mentioned conditions and their concentrations were determined from the calibration plot.

**Table 1: Hierarchical classification of *T.Telescopium* and *B.spinosa***

<i>T.telescopium</i>	<i>B.spinosa</i>
Kingdom : Animalia	Kingdom : Animalia
Phylum : Mollusca	Phylum : Mollusca
Class : Gastropoda	Class : Gastropoda
Superfamily : Cerithioidea	Superfamily : Tonnoidea
Family : Potamididae	Family : Bursidae
Genus : Telescopium	Genus : Bursa
Species : <i>T.telescopium</i>	Species : <i>spinosa</i>



**Fig.1(a):-*T.telescopium*      Fig.1(b):-*B.spinosa***

**Figure 1:- Species under study**

## RESULT AND DISCUSSION

Principal fatty acids in lipid fractions of the two gastropod species *B. spinosa* and *T. telescopium* were palmitic acid (6.11% (in *B. spinosa*), 20.16% (in *T. telescopium*)), stearic acid (4.76-6.88%), oleic acid (6.13% (in *B. spinosa*), 19.11% (in *T. telescopium*)) and 5,8,11,14-eicosatetraenoic acid (5.36% (in *T. telescopium*), 5.71% (in *B. spinosa*)) (Table 2). This result showed a resemblance with the observations of<sup>19,20,21</sup>. Major fatty acid compositions observed in all these species were the same. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were the major polyunsaturated fatty acids (PUFAs) observed in *T. telescopium* ( $5.97 \pm 1.43$  and  $8.30 \pm 3.21$  respectively). Approximately, 50% of the total fatty acid fraction was covered by PUFAs in *B. spinosa*. Out of which, 44% comprised of docosapentaenoic acid (DPA). Marine members from the Molluscan class gastropods have been found to contain 18:1n-9, 20:4n-6, 20:5n-3 and 22:5n-3 as the major fatty acids<sup>22,17</sup>. C16:0 and C18:0 were the major saturated fatty acids (SFAs) observed both in *T. telescopium* and *B. spinosa*, while C14:0 was observed only in *B. spinosa* species. C20:0 and C22:0 were observed in trace amount. Odd chain fatty acids such as pentadecanoic acid (C15:0), heptadecanoic acid (C17:0) and nonadecanoic acid (C19:0) were present in both the species. Tridecanoic acid (C13:0) (1.88%) was observed only in *T. telescopium*. Collectively, the SFAs were present in much greater quantity than polyunsaturated ones in *T. telescopium*, while the reverse was observed for *B. spinosa* (Fig. 2). Among the SFAs, hexadecanoic acid occurred in major quantity among all the fatty acids quantified. This is in good agreement with previous reports<sup>23,10</sup> that C16 fatty acid is the main fatty acids in molluscs and in all tropic levels too. From the literatures, it was clear that C13 and C14 were especially active in antisporeulation<sup>24,8</sup>. According to<sup>25</sup>, twenty-two SFAs were found in *Leuconia johnstoni* species with relative abundance 59.1%. Studies on the lipids of adult molluscs show that palmitic (16:0) and stearic (18:0) acids were the dominant fatty acids<sup>26,27,28</sup>. SFAs with an uneven chain length (nonadecylic, heptadecylic, pentadecylic and tridecyllic acids) were detected in the extracts of both *T. telescopium* and *B. spinosa*, and the same have been previously reported from molluscs tissue<sup>26,27</sup>.

Branched SFAs found were 4, 8, 12- methyl tridecanoic acid (TMTD), isopentadecanoic acid (iC15:0), anti isopentadecanoic acid (a-C15:0), isoheptadecanoic acid (iC17:0), and anti isoheptadecanoic acid (a-C17:0) (Table 2). Out of these detected branched chain fatty acids, TMTD and iC17:0 were major in *T. telescopium*. Previous studies have reported that TMTD was produced through the degradation of dietary chlorophyll<sup>22</sup>. The amount of 4,8,12 TMTD was observed as more abundant in *T. telescopium*. 4, 8, 12 TMTD origins from the herbivore chiton's diet and is derived

from phytol and chloroplast membrane lipids. So it is possible that internal organs contribute the metabolism of the

se fatty acids. Previous studies had reported that 16-methyl-octadecanoic acid (ai-19:0) as a major FA in several calcareous sponge species mostly of the calcinea subclass<sup>30</sup> whereas in low amounts were found in both *T. telescopium* and *B. spinosa*. 2-Hydroxy fatty acid was identified by GC-MS as 2-hydroxy hexadecanoic acid (0.21%) in *B. spinosa*, while it was absent in *T. telescopium*. According to 12c, 2-hydroxy fatty acids are generally derived from structural components of membranes called spingolipids. 2-hydroxy hexadecanoic and heptadecanoic acids were reported in some species of mussels.

Carbon chain	Fatty acid	<i>T.telescopium</i>	<i>B.spinosa</i>
C13:0	Tridecylic acid	1.83 ± 0.3	
C14:0	Myristic acid		1.14 ± 0.12
C15:0	Pentadecylic acid	1.82 ± 0.12	0.70 ± 0.19
C16:0	Palmitic acid	20.16 ± 0.93	6.11 ± 1.21
C17:0	Margaric acid	2.82 ± 0.17	2.34 ± 0.31
C18:0	Stearic acid	6.88 ± 0.3	4.76 ± 0.34
C19:0	Nonadecylic acid	0.34 ± 0.11	0.23 ± 0.11
C20:0	Arachidic acid	0.49 ± 0.13	
C22:0	Behenic acid		0.12 ± 0.21
C23:0	Tricosylic acid		0.09 ± 0.06
4,8,12-Me C13	4,8,12-trimethyl tridecanoic acid	1.82 ± 0.22	
iC15:0	Iso pentadecanoic acid	0.48 ± 0.12	0.14 ± 0.02
a-C15:0	Antiiso pentadecanoic acid	0.19 ± 0.09	
iC17:0	Iso heptadecanoic acid	7.13 ± 0.12	0.13 ± 0.11
a-C17:0	Antiiso heptadecanoic acid		
C16:1n3	13-hexadecenoic acid	0.93 ± 0.11	0.36 ± 0.18
C16:1n7	Palmitoleic acid		
C18:1n9	Oleic acid	6.10 ± 0.18	0.91 ± 0.17
C18:1n6	13-octadecenoic acid	6.13 ± 0.36	19.11 ± 0.21
C19:1n7	10-nonadecenoic acid	0.39 ± 0.14	
C20:1n5	5-eicosenoic acid	7.74 ± 0.23	
C20:1n9	13-eicosenoic acid	0.92 ± 0.31	0.34 ± 0.08
C18:2n4	11,14-octadeca dienoic acid	2.53 ± 0.35	0.39 ± 0.22
C18:2n6	Linolenic acid	0.73 ± 0.12	0.29 ± 0.10
C20:2n7	10,13-eicosa dienoic acid	1.09 ± 0.65	1.02 ± 0.99
5,13-C22:2	5,13-docosa dienoic acid	0.72 ± 0.21	
C22:2n6	4,7,10,13,16-docosa pentaenoic acid	1.39 ± 0.54	
C20:3n6	Linolenic acid	0.96 ± 0.10	2.40 ± 0.89
C18:4n4	Arachidonic acid	5.36 ± 0.76	
C22:4n6	Docosa tetraenoic acid	2.67 ± 0.87	5.71 ± 0.19
C20:5n3	Eicosa pentaenoic acid	5.97 ± 0.43	1.62 ± 0.78
C22:5n3	Docosa pentaenoic acid	1.55 ± 0.98	44.08 ± 0.88
C22:5n6	Obsbond acid	0.98 ± 0.21	
C22:6n3	Docosa hexaenoic acid	8.30 ± 0.21	1.61 ± 0.76

Table 2: Fatty acid fractions in *T. telescopium* and *B. spinosa*

13-Hexadecenoic acid (C16:1n3), oleic acid (C18:1n9), 13- octadecenoic acid (C18:1n6), 5-eicosenoic acid (C20:1n5), 10- nonadecenoic acid (C19:1n7) and 13-eicosenoic acid (C20:1n9) were the mono unsaturated fatty acids (MUFAs) present in *T. telescopium* species (Fig. 3). Further, the fatty acids, C16:1n3, C19:1n7 and C20:1n9 were observed in very low levels (< 1%). Oleic acid (C18:1n9) was the predominant MUFAs present in *B. spinosa* species, while palmito oleic acid (C16:1n7) and 13-eicosenoic acid methyl ester (C20:1n9) were present in very low quantity. Previously, some 2-methyl branched unsaturated FAs have already been isolated but only from siliceous marine sponges. Studies have shown that MUFAs help to reduce blood cholesterol levels and protect against heart disease, so these type of fatty acids coming under the category of good fat<sup>8</sup>. In the present study, MUFAs was recorded at 20% for both species, among the MUFAs, C18:1 n9 (19% for *B. spinosa* and 7% for *T. telescopium*) was dominant. This result shows resemblance with the reported data of MUFAs from oceanic gastropod *Xancus pyrum*. Previous reports obtained from the fatty acid profile of gastropods shows good agreement with the present study, that they contain C18:1 as major MUFA.

It was shown that marine molluscs are rich sources of 20:5n3 fatty acids, while 22:6n3 is less in amount. The proportion of these two type of n3 fatty acids in *T. telescopium* and *B. spinosa* differed significantly. The concentration of C20:5n3 was higher in *B. spinosa*, species than in *T. telescopium*. C22:6n3 was present in *B. spinosa* while it was absent in *T. telescopium* (Table. 2). The proportions of n3-PUFA were particularly high in muscle tissue of megangulus variety<sup>21</sup>. DPA (C22:5n3) was the predominant PUFA present in *B. spinosa* species (44%) (Fig.4), whereas low in *T. telescopium*, species. The proportions of n3 PUFA were particularly rich in *B. spinosa* constituting 45% of the total fatty acids (Fig.4). But in the case of *T. telescopium* only below 15% and < 5% constitute the n3 and n6 PUFA respectively. Compared to the other fatty acids, the concentrations of PUFAs were low in *T. telescopium* whereas in the case of *B. spinosa*, PUFAs were the predominant fatty acids. n3 fatty acids are vital for normal metabolism.

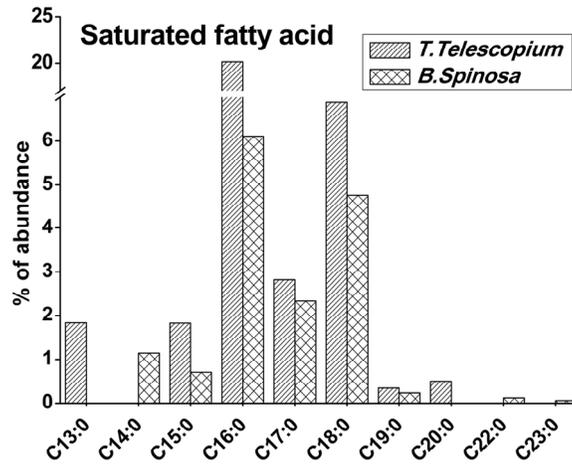


Figure 2: Percentage composition of saturated fatty acids

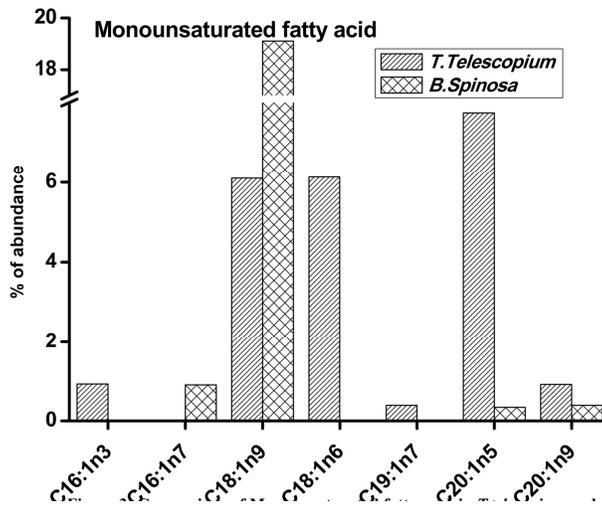


Figure 3: Percentage composition of mono unsaturated fatty acids

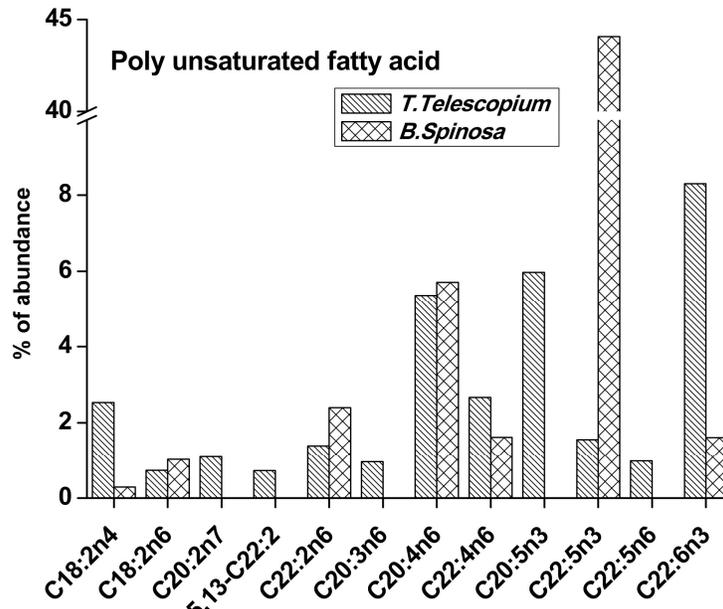


Figure 4: Percentage composition of poly unsaturated fatty acids

PUFA tends to reduce the blood cholesterol levels and is considered as a “good” fat. From the reported literatures, it was clear that PUFAs with 20 and 22 carbons and more than three double bonds, which are sparingly, or not at all, biosynthesized by bivalves, are essential for survival, growth and reproduction of the molluscs<sup>34,35,8</sup>. In the present study, *T. telescopium* contains C22:6n3 (8%) and C20:5n3 (6%) of the total fatty acids and *B. spinosa* contains C22:5n3 (44%). These 20- or 22-carbon PUFAs, notably C22:6n3, C20:5n3 and C20:4n6, are of particular importance in membrane phospholipids. According to, some of the PUFAs are found to be essential for the healthier growth and survival of fin fishes.

Both 18:3n3 and 18:2n6 fatty acids were reported from another cultured warm water fish, eel (*Anguilla japonica*) at the level 0.5%<sup>36</sup> which is similar to the results obtained from this study (0.7%-1.02%). According to<sup>37</sup>, patients with neuropathy diseases have the deficiency of n3 fatty acids. Reported describes that in some of the cases, n3 fatty acids supplementation had positive effects on the neurological symptoms. Hence, dietary intakes of n3 fatty acids are very effective towards bronchial asthma, lupus erythematosus multiple sclerosis, psoriasis and kidney diseases<sup>38</sup>. In *B. spinosa*, n3 fatty acids contributed 45% of total fatty acids, so it can be suggested the inclusion of such animal in normal diet of patients suffering from neurological diseases. C20:4n6 (characteristic to freshwater mollusc) and C20:5n3 (characteristic to marine mollusc) are important mediators in basic physiological functions, ion regulation, renal function and reproductive process in molluscs<sup>3</sup>. Insignificant amounts of C20:4n6 fatty acids were reported in the species studied, whereas the percentage of C20:5n3 was reported at high level in *T. telescopium*. Reported data give the knowledge that C20:4n6 is mostly associated with reproductive process and not with growth. The reported data of PUFAs in the mantle of *T. telescopium* were found to be high<sup>39,5</sup>. According to<sup>5</sup>, marine molluscs possess very low level of C20:4n6 acid, but in fresh water bivalves, its amount was relatively high. There is experimental evidence that marine n3 PUFA reduces the risk of breast cancer but this is not conclusive. Consumption of EPA and DHA stimulate blood circulation, increase the breakdown of fibrin, a compound involved in clot and scar formation and, in addition, may reduce the blood pressure. So these fatty acids can have medicinal applications for certain circulatory problem such as varicose veins. *T. telescopium* contains 5% EPA and 8% DHA of the total PUFAs. DHA is normally one of the most abundant fatty acids in marine animals, including marine molluscs. Comparable results were obtained in the present study. Previous reports also show that n3 fatty acids reduce blood triglyceride levels, regular intake of this may reduce the risk of primary and secondary heart attack.

Non methylene interrupted (NMI) fatty acids were detected in both species. C18:2n6 (0.73%), C18:4n4 (5.36%), C20:3n6 (0.96%), 5,13-C22:2 (0.72%), C22:5n6 (0.98%), and C22:4n6

(2.67%) were the NMI fatty acids detected in *T. telescopium*. C22:4n6 and C18:4n4 were present both in *T. telescopium* and *B. spinosa* species (Table. 2). From the above NMI fatty acids, C18:4n4 was predominant in these two species. This result showed good agreement with the results of gastropods obtained from Mediterranean Sea and Red Sea<sup>17</sup>. NMI fatty acids are usually present in many marine invertebrates such as mussels, oysters and gastropods<sup>21</sup>, but the functions are not fully understood.

## CONCLUSION

Conclusively, data from the present study demonstrated that *T. telescopium* and *B. spinosa* contained iC17:0, C16:0, C18:0, C18:1n9, C18:1n13, C20:1n5, C18:4n4, C22:4n6, C20:5n3, C22:5n3 and C22:6n3 fatty acids. C16:0, C18:1n9, C18:4n4, C22:5n3, C22:6n3 were found to be predominant. The relative abundance of PUFAs and MUFAs were high. Consumption of EPA and DHA stimulate blood circulation, and these FA can be suggested as remedy for certain circulatory problem such as varicose veins. *T. telescopium* contains 5% EPA and 8% DHA of the total PUFAs. But in *B. spinosa* EPA was absent and the amount of DHA was only 1%. *B. spinosa* contains 44% DPA, has pharmacological value. On the basis of these results, *T. telescopium* and *B. spinosa* are good sources of some pharmacologically important fatty acids. Marine molluscs cannot synthesise n3 and n6 fatty acids in their body, they earn these fatty acids from the normal diet such as phytoplankton, sea weeds and algae. So their n6/n3 values vary considerably with species. The total n3 ( $\Sigma n3$ ) fatty acids in both the species were higher than the total n6 ( $\Sigma n6$ ) fatty acids, and the  $\Sigma n3/\Sigma n6$  ratio was found to be 1.30 in *T. telescopium* and 1.73 in *B. spinosa* (Table 2).

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